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Borreliae, Human Relapsing Fever, and Parasite-Vector-Host Relationships

OSCAR FELSENFELD

Walter Reed Army Institute of Research, Washington, D.C.

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INTRODUCTION

Borreliae pathogenic to man are the causative agents of relapsing fever and its varieties known as tick fever, ghurib gez (Iran), carapata (Africa), kimputu, gorgoya (South America), bilious typhoid, fowl nest fever (China), and vagabond fever (Spain).

The relapsing fevers may be divided into: (i) louse-borne, which is usually epidemic, and (ii) tick-borne, which is mostly endemic. Exceptions, however, occur; e.g., in Abyssinia, Peru, and China, the louse-borne type became endemic.

Human *Borrelia* infections are or were seen all over the world, except in Australia, New Zealand, and Oceania. Only one imported case was recorded in that area (159).

The appearance of louse-borne *Borrelia* infections is usually connected with crowding, poor housing, undernourishment, lack of cleanliness, and little change of clothing, as is the rule during disasters like war, fire, earthquake, flood, and famine.

Europe, Asia, and Africa suffered from extensive louse-borne *Borrelia* infections which originated near Fezzan during and shortly after World War I and World War II.

Tick-borne *Borrelia* infections remain mostly restricted to the vicinity in which *Ornithodorus* lives and do not spread further than the infected arthropod host is moving. The contacts of the ticks with man are few; thus, the human infection rate is low. Since inhabitants of areas where ticks are prevalent may have acquired a certain degree of immunity during childhood, it is

chiefly the outsider who becomes ill in such places. Usually soldiers, hunters, field workers, and tourists are the victims of tick-borne relapsing fever. Sometimes the tick vector is associated with domestic animals, like sheep in the Kashmir (169), creating a hazard to their tenders, or with fowl kept in living huts, as in East Africa (284).

It is the purpose of this contribution to review critically current information on borreliae, their relationship to insect vectors, the pathology of experimental infection, and the immunology and laboratory diagnosis of human relapsing fever.

TAXONOMY

The genus *Borrelia* Swellengrebel 1907 belongs in the family Treponemataceae Robinson 1948 of the order Spirochaetales Buchanan 1918. As a result of numerous considerations, the human-pathogenic members of this genus were (and still are) often designated by different names, such as *Spirillum*, *Spirochaeta*, *Spirocheta*, *Spirochaete*, *Spironema*, *Treponema*, *Protomycetum*, and others. Practical differentiation of the organisms belonging in the family Treponemataceae was suggested by Davis (95) and Hindle (157, 158, 159).

The classification of the strains within the genus *Borrelia* is greatly hampered by their immunological mutations in the host during the bouts of relapsing fever (175), and also under the influence of other environmental factors (136), including the seasons (31). Because of the close relationship of arthropod species to specific *Borrelia* strains, the "one vector-one species" concept has been developed, and numerous authors (93, 96, 160, 167, 280) have strongly advocated the naming of borreliae according to their vectors. Since louse-borne strains differ from tick-borne strains not only epidemiologically but also in animal experiments, the presently used separation of louse-borne and tick-borne relapsing fever has been maintained throughout this report.

Table 1 enumerates the relapsing-fever *Borrelia* strains well defined at present, together with their tick hosts. Borreliae causing animal disease only are not included. However, strains suspected of being the causative agents of human disease, although they have not been fully proved pathogenic for man, are listed.

MORPHOLOGY

Borreliae are usually 10 to 20 μ long and 0.2 to 0.3 μ wide, but their length can range from 8 to 40 μ and their width from 0.2 to 0.5 μ (158, 159). They have 3 to 6, and sometimes even 10, spirals; the average is 5 to 7. The amplitude of the spirals or "waves" averages 1 μ. These measurements are highly dependent on the stain used on the organisms. The strains cannot be differentiated by size (247). Manson and Thornton (200) saw predominantly short forms in East and West Africa; Hindle (159) found elongated borreliae in Central Africa. In Indochina (208) and in Addis Ababa during the Italian occupation (259), slender as well as very short forms were recorded. Just before the crisis in relapsing fever, when the number of borreliae in the blood of the patient decreases, bizarre forms appear. These forms are often less motile and accumulate in rosettes, owing to adhesin activity (*vide infra*).

Variations in morphology often appear when the organisms are transferred to a heterologous animal. One strain of *B. recurrentis* showed abundant, irregular, bent, ringlike, small and thin forms in animals infected with human blood in which such forms were not seen (271). The morphology of borreliae may also vary during subsequent attacks in the same patient (269). These morphological changes are due to the action of antibodies.

Borreliae have no undulating membrane, but they do have a contractile axial filament of protoplasmic nature covered by a periplast. This filament is said to be essential for the penetrating ability of *Borrelia*. Numerous flagella were observed in a substrain of *B. novyi* which had been perpetuated in the laboratory for many years, the origin of which was spurious (190). Optical microscopy shows only one short terminal filament (158), which is especially apparent after division.

Babudieri (12, 13) and Babudieri and Bocciarelli (15) examined *B. recurrentis* and the novyi strain with an electron microscope. They found one end rounded and the other pointed during the resting stage. The protoplasm was homogenous with few vacuoles and granules. These authors did not see an axial filament, but they verified the presence of an undulating membrane along the entire length of the organisms; this membrane could be macerated into fibers. Similar structures were seen in trypanosomata.

Kawata (173) worked with *B. duttonii*. He saw a distinct cell membrane, threadlike fibrous and granular structures similar to the nuclear apparatus of bacteria, and dense areas resembling nuclear sites. He (174) believed that *B. duttonii* has a foamy envelope which is dissolved in sodium deoxycholate. Kawata observed 20 to 25 fibrils on the surface of the cell wall. Electron microscopy revealed neither mitochondria nor a limiting membrane between the cytoplasm and the nuclear zone. Lofgren and Soule (198), ex-

TABLE 1. *Borreliae and their vectors*[a]

Group	*Borrelia*	Vector
Louse-borne	*B. recurrentis* Lebert 1874	*Pediculus humanus* Linnaeus 1758
	(*B. obermeieri* Cohn 1875)	
	[*B. berbera* Sergent and Foley 1910]	
	[*B. carteri* Mackie 1907]	
	[*B. novyi* Schellack 1907]	
	[*B. kochi* Novy 1907 = *B. rossi* Nuttal 1908]	
	[*B. aegyptica*][b]	
Tick-borne		*Ornithodorus*[c] Koch 1844
		(*Ornithodoros*)[c]
	B. hispanica de Buen 1926	*O. erraticus erraticus* Lucas 1849
	[*B.* hispanica var. *maroccana* Breeze 1909]	("large" form of *O. erraticus*)
	[*B. hispanica* var. *mansouria*][b]	(*O. erraticus* var. *maroccanus*)[b]
		(*O. maroccanus* Vela 1919)
		(*O. miliaris*)[b]
	B. crocidurae Læger 1917 group:[c]	*O. erraticus sonrai* Savtet, Marnette, and Nitowsky 1944
	B. crocidurae Leger 1917	
	B. microti Rafyi 1947	("small form" of *O. erraticus*)
	B. meronesi Blanc and Maurice 1947	
	B. dipodilli Heisch 1950	
	B. duttoni Novy and Knapp 1906	*O. moubata* Murray 1877 (at least four subtypes)
		O. savignyi Audoin 1827[d]
	B. granigeri Heisch 1953[e]	*O. granigeri* Heisch 1953
	B. persica Dschunkowsky 1913	*O. tholozani* Laboulbène and Mèguin 1882[e]
	[*B. uzbekistana* Pikoul 1928]	(*O. papillipes* Birula 1895)
	[*B. sogdiana* Nicolle and Anderson 1928]	(*O. crossi* Brumpt 1929)
	[*B. babylonensis* Brumpt 1939]	[*O. asperus* Warburton 1918 = var. *babylonensis*]
		[*O. persepolensis*][b]
		[*O. pavlovskyi*][b]
	B. latishevyi Sofiev 1941	*O. tartakovskyi* Olenev 1931
		O. neerensis Pavlovskyi[f]
	B. caucasica Maravachvili 1945	*O. verrucosus* Olenev, Zasukhin, and Fenik 1934
	B. turkmenica[b, c]	*O. cholodkovskyi* Pavlovskii 1930
	B. venezuelensis Brumpt 1921	*O. rudis* Karsch 1880[g]
	(*B. neotropicalis* Bates and St. John 1922)	(*O. venezuelensis* Brumpt 1921)
	1. Unnamed	
	2. *B. mazzottii* Mazzotti 1953	*O. talaje* Guérin-Ménevillel 1849[g]
	B. parkeri Davis 1942	*O. parkeri* Cooley 1936
	B. turicatae Brumpt 1933	*O. turicata* Dugès 1876
	B. hermsi Davis 1942	*O. hermsi* Wheeler, Herms, and Meyer 1935
	B. brasiliensis Davis 1952	*O. brasiliensis* Aragão-Beaurepaire 1923

[a] Names enclosed in parentheses are synonyms; those in brackets are possibly subspecies but perhaps only synonyms.
[b] Description not clear enough to warrant full acceptance.
[c] Nomenclature not settled.
[d] Not established as vector.
[e] Probably has subtypes.
[f] *O. neerensis* is also supposed to carry *B. latishevyi*.
[g] Pathogenicity for man not fully established.

amining the *B. norvi*, found smooth cell walls and a granulated or motyled protoplasm; pointed tips, sometimes with a heavy terminal filament, were frequently seen. Flagella-like fibers, swelling, and granulation with free or attached heavy granules were produced. Möllbert (210) observed a fibrillar band consisting of 18 to 20 single fibrils connected with an activator membrane.

These findings are not wholly consistent with observations made under an optical microscope, and further research with the use of different strains is desirable.

Borreliae are flexible organisms. Dobell (108) pointed out that anterioposterior polarity is completely absent in them. Consequently, movement is possible in the direction of either end. Borreliae move back and forth over a distance of two to three times their length. Their locomotion may be (158, 159) (i) corkscrew-like; (ii) wavelike, forwards and backwards; or (iii) by lateral bending and looping.

Ackermann and Protasov (1) stated that the type of movement depends on external conditions. In guinea pig blood, their Central Asian strain showed serpentine as well as rotary movement, and formed rings as a protective measure.

Apparently the movement of borreliae, like their morphology, may vary under the influence of antibodies.

Borreliae multiply by transverse division. During this process, the two halves become somewhat entwined preparatory to final separation (174), which may give the impression of longitudinal division. The division takes 10 to 15 min. Unusually long forms, sometimes seen in blood and described in Central African relapsing fever by Hindle (159), are the result of end-to-end attachment after division. During fission, the organism constricts in the middle, and the periplast can be seen as a threadlike structure. This structure may be considered a flagellum after the process of division has been completed.

Borreliae are seldom found in the blood during intervals between relapses. The cerebrospinal fluid remains infectious in experimental animals after the organisms have disappeared from the blood, but here, as in ticks and lice during the period when borreliae are leaving the stomach and reappear in the celomic cavity (so-called "negative phase"), organisms are seldom observed. Granules of unexplained origin may be found in body fluids and some organs during the entire disease. Thus, theories have been published in which a life cycle of *Borrelia*, including invisible or granular forms, was described.

So-called metacyclic forms were studied by Baltazard et al. (23). These workers suggested that "invisible" forms, either "filtrable" or "granular," circulate in the blood (28). Chorine et al. (77) found only a few small, coccoid or granular forms of *Borrelia* passing through bacteriological filters. Perhaps granular and small forms (including fragments) pass through filters when the pressure is high enough, but the existence of filtrable or invisible forms of *Borrelia* has not been proved as yet.

When borreliae break off into smaller forms during the crisis of an attack, degenerative fragments and granules can be seen (287). Johnstone (167) classified granules into two groups: one group representing part of the life cycle of *Borrelia*, and the other originating from a degenerative process. Burgdorfer (58), working with *Ornithodorus moubata*, found a reduction in size of borreliae in passage, but he could not confirm their role in a supposed life cycle. Heisch et al. (148, 149, 153) observed coarse, irregular, structureless granules in lice, which could not be proved to be a developmental stage but had to be considered breakdown products.

Borreliae certainly undergo morphological variations, but it is questionable whether they have a life cycle which includes atypical and granular forms. Fluorescence microscopy permits observation of granular forms, which appear to be breakdown products and involution forms rather than evolutionary stages (Felsenfeld, *unpublished data*).

STAINING

Borreliae can be stained with practically any aniline dye (59), but acid dyes are most effective (285). Du (109) dehemoglobinized the slides with 6% acetic acid in 95% ethyl alcohol for 5 sec; he then stained them with carbol fuchsin for 1 min. This is a simple procedure and is feasible also for thick smears.

Silver stains are not easy to use, and there is a danger of precipitations which may be misinterpreted as granular forms. Moreover, silver impregnation changes the morphology of the organisms.

Routine blood stains (Giemsa, Romanowski, Wright, Leishman) are often used. Prolonged staining is recommended.

Coles (84) also recommended a prolonged (12 to 48 hr) staining of air-dried and ethyl alcohol-fixed smears. After washing off the Giemsa stain, orange tannin was poured on the slides for 15 min, for differentiation. A final washing followed.

Our method (Felsenfeld, *unpublished data*) consists of staining with Wright's stain followed by the application of a 1% crystal violet solution for 10 to 30 sec.

Vago (275) introduced Mercurochrome in the staining of *Borrelia*. Mercurochrome alone does not stain intensively enough and is not taken up uniformly by the organisms in the presence of serum.

Young (295), in an effort to avoid distortion of the organisms due to fixation, stained the organisms for 3 min with a concentrated aqueous solution of Mercurochrome, rinsed them with distilled water, applied concentrated aqueous methyl violet, washed them with distilled water, and air-dried the smears.

A method for concentrating borreliae was described by Simons (261). It is based on the observation that borreliae are not dissolved by 10% bile for several weeks. Thus, 2 ml of 10% sodium taurocholate in saline were mixed with 1 ml of saturated methylene blue in saline. This mixture will keep for about 1 month. Two to four loopfuls of this solution were added to an equal amount of blood, and a smear was made and covered with a cover slip. To examine larger amounts of blood, to each 2 ml of blood 1 ml of sodium citrate solution and 0.5 ml of the taurocholate solution were added, the mixture was centrifuged, and the sediment was examined with dark-field optics.

BIOCHEMISTRY

Borreliae always move to the cathode in an electrophoretic apparatus (120). Borreliae, although often considered relatives of *Trypanosoma*, differ from the latter by not utilizing oxygen. Oxygen, however, is not toxic for borreliae (119, 206). The utilization of dextrose was found to be glycolytic, its extent depending on the number of borreliae (119). Cell-free extracts and homogenates showed enzymes of the Embden-Meyerhof pathway of glycolysis (122). *B. novyi* utilized large amounts of dextrose at 37 C; about 65% accumulated as lactic acid and about 10% was oxidized completely to carbon dioxide and water (206, 253).

RESISTANCE

In wet preparations, sealed with a cover glass, borreliae survive for about 1 day at room temperature. Hindle (158, 159) stated that known human pathogenic borreliae were killed when exposed to 50 C for 30 min. The organisms remain alive in citrated blood for 3 months at 0 to 2 C. Central Asian strains lived as long as 100 days, but their number decreases progressively during this period. Beck (34) observed the survival of Californian strains in refrigerated sheep blood for 195 days but in frozen tissue only for a few days. Bourgain (49, 50) worked with *B. persica*. His strain remained alive for 19 days at 4 C, 7 days at 11 to 15 C, and 4 days at 37 C. In isolated organs, it survived for 7 days at 0 to 8 C, but in cadavers kept at room temperature it survived for only 4 days. The strain died, however, at −15 or −20 C within 2 days. On the other hand, Weyer and Mooser (288) were able to keep several *Borrelia* strains alive for 1 year at −72 C. Freezing and thawing the organisms three or more times destroyed them (197). These findings show the great variability of the survival time of borreliae.

Borreliae cannot withstand desiccation, but light used to illuminate laboratories does not affect them (159). Borreliae survive better in a slightly alkaline environment (179), and they are susceptible to many chemical agents.

Sulfonamides tested against *B. recurrentis* in rats proved ineffective (164). Only a feeble response was noted when penicillin was used against the *B. novyi* in rats (88). There was no synergism against *B. duttonii* in mice between the arsenicals, which were widely used in the preantibiotic era, and penicillin (68). Combinations of penicillin and streptomycin did not destroy *B. duttonii* in mice (37). However, large doses of streptomycin were effective against the same organism in rats (193).

Streptomycin and chloramphenicol had some effect, and neomycin gave excellent results in experiments with *B. novyi* (116, 117). In guinea pigs infected with *B. hispanica*, chloramphenicol was effective, chlortetracycline gave excellent results, and oxytetracycline was the most efficacious (63). In mice infected with *B. novyi*, five times greater doses of chlortetracycline had to be given orally than intra-abdominally to effect cure (144). Oxytetracycline quickly cleared the blood of rats infected with *B. duttonii*, but brain infection and relapses were not prevented (152); chlortetracycline, given prophylactically, delayed but did not prevent disease (151). The lack of prophylactic value of oxytetracycline in rats with *B. persica* was also observed by Adler et al. (6), who emphasized the therapeutic efficacy of this antibiotic.

Tetracycline killed borreliae when given early in *B. hispanica* and *B. recurrentis* infections (105). With Central Asian strains, albomycin was found to be a highly active therapeutic agent (278).

Discrepancies in pharmacological reports may be due to strain variance and to the fact that some authors consider the disappearance of the borreliae the criterion of therapeutic success, whereas others regard the immobilization of these organisms as evidence of success. The mode of action of antibiotics on *Borrelia* has not been explored as yet, nor has the influence of such agents on the formation of antibodies been investigated.

CULTURE METHODS

In Vitro

None of the culture methods tested has assured the multiplication of *Borrelia*. Present methods are designed to maintain the organisms alive rather than to grow borreliae.

Of the many media recommended, the following are of interest.

Chorine and Crougue (76) mixed peptone, water, fresh rabbit serum, and laked or defibrinated human blood. At 28 to 30 C, slow growth was observed in this fluid.

Wolman and Wolman (293) coagulated 1 ml of egg albumin at 80 C in culture tubes, and added 10 ml of a mixture consisting of 1 part human ascitic fluid, 1 part buffer solution (*p*H 7.8), 2 parts saline, and 1 part 1% dextrose. Liquid paraffin was added to cover the medium. Heating at 56 C for 1 hr each day for 3 consecutive days was used for sterilization. *B. recurrentis* survived in this medium for 8 months at 0 to 12 C. At 42 to 45 C, the organisms still remained active, but they lost their virulence for animals when kept at higher temperatures. *Borrelia* strains collected in Abyssinia from patients with severe illness survived even longer in this medium.

In Developing Chick Embryos

Chabaud (69), in experiments with *B. duttonii*, used defibrinated blood and inoculated the chorioallantoic membrane of 4- to 5-day-old eggs. The embryos died on the 6th or 7th day after inoculation. The survival time was reduced to 40 hr after repeated egg-to-egg passages. Chabaud also found that defibrinated blood gave better results than citrated blood.

Ong (225, 226) did not observe changes in the chorioallantoic membrane with his strain of *B. duttonii*, and the embryos stayed alive. Serial passage increased the motility but not the virulence of the strain. Borreliae appeared in the blood of the chicks if the embryo was infected 2 to 3 days before hatching, and circulated in the chicken for about 5 days. They disappeared, however, within hours from the blood of chicks newly hatched from *Borrelia*-free eggs when inoculated with *B. duttonii* after hatching. This is in accordance with the observations of Rodhain and van den Berghe (246), who stated that *Borrelia* strains, such as *B. gallinarum*, growing in adult fowl, do not multiply in the developing chick embryo and vice versa.

Bajramova (16) reported success in culturing tick-borne Asian borreliae in developing chick embryos.

Chen (73) inoculated *B. recurrentis* into the yolk sac of developing chick embryos. The maximal number of borreliae was observed 5 days after inoculation. When the embryos died, the borreliae disintegrated.

Hallauer and Kuhn (143) worked with a European strain of *B. recurrentis*; 9- to 10-day-old eggs were used. The organisms appeared in the blood of the embryos in 2 to 3 days, and the embryos died in 3 to 5 days. No loss of virulence was observed in 35 passages. Bianchi (36) obtained similar results.

In experiments with *B. recurrentis* from the 1943–1945 epidemic in North Africa, Baltazet (18) encountered difficulties in establishing and passaging this strain, but final results were satisfactory. The borreliae showed degenerative changes and lost their pathogenicity for mice during egg passage. A mixture of allantoic fluid, embryo blood, and some citrate was used to propagate the strain from egg to egg. The organisms appeared in the blood of the chick embryos 3 to 4 days after inoculation.

Thus, significant differences were observed in the growth of *Borrelia* in developing chick embryos. The age of the egg to be inoculated, the mode of inoculation, and the optimal incubation temperature remain to be established for many strains.

Preservation of Borreliae in Rodents

Pampana (227) described an efficient method for the use of laboratory animal brains for the preservation of *Borrelia*. Within 2 to 6 months after infection, depending on the strain, the guinea pigs were killed with chloroform. The brains were washed with saline, emulsified, and injected intraperitoneally or subcutaneously into fresh guinea pigs. The incubation time was 6 to 12 days, or sometimes longer. *B. hispanica* could be recovered from brains of the infected guinea pigs for at least 2.5 months (252), and often after 3 years (257). Guinea pigs can be used only for the maintenance of borreliae to which they are susceptible but which do not cause fatal disease in them. Guinea pigs are unreliable for the preservation of *B. persica*. This organism may survive in the guinea pig brain for 222 days or may disappear after 45 days. Variations among species are considerable (236). *B. hispanica* was found alive in rat brains after 17 months. Rats, however, were not feasible for survival experiments with the Manchurian strains of *B. recurrentis* (270). Borreliae do not survive long in mouse brains (270).

This method of *Borrelia* preservation has to be tested with each individual organism and in each animal colony before it can be used for practical purposes.

Preservation of Borreliae in Ticks

The long life span of ticks predestinates them for use as laboratory media for the maintenance of *Borrelia*. *O. tholozani*, for example, may live as long as 25 years, and was able to transmit *Borrelia* after 12 years (231). Fasting nymphs remained infective for 10 years (51). *O. tholozani* was found feasible also for the maintenance of Central Asian *Borrelia* strains (228). *O. erraticus* can be used for the preservation of *B. hispanica* even at 5 to 7 C for many weeks (54). *O. turicata*, starved for 5 years, was still able to transmit *B. turicatae*.

However, not all members of a tick colony are equally susceptible to *Borrelia* infestations. There is also a certain mortality in every tick colony. Nevertheless, this method is most feasible for the preservation of several *Borrelia* strains in the laboratory.

RELATIONSHIP OF BORRELIAE TO THEIR INSECT VECTORS

Lice

The human louse, *Pediculus pediculus humanus*, is the transmitting agent of *B. recurrentis*. While both var. *vestimenti* and var. *capitis* proved to be effective transmitters of *B. recurrentis*, the crab or pubic louse (*Phthirus pubis*) is not a vector (60, 72, 163).

Lice are more easily transferred from man to man during the winter, because they prevail in heavily clothed people. Thus, in hot and humid Central Africa the naked inhabitants escape lice. When temperature and moisture surpass the optimum for lice, the mobility of these insects is reduced. They lay eggs and separate from man; then they begin to die.

One louse takes up about 1 mg of blood at one feeding (60). Heisch et al. (135) found that this meal has to contain at least one or two borreliae per oil immersion field to be infective. Thus, lice will seldom become infected during remissions when few or no borreliae circulate in the blood. Only about 12 to 17%, of the lice fed on patients during an attack of relapsing fever transmitted the disease (222, 223).

The borreliae enter the midgut of the louse. Mechanical transfer of borreliae by louse bite or by louse feces is possible only within a few hours after feeding on infected persons. Many of the ingested borreliae perish in the louse. Their rate of survival is proportionate to their resistance to the digestive juices of the louse (147). The remaining borreliae lose their motility, and disappear from the gut in about 1 day. A "negative phase" ensues during which the borreliae are present as granules, which can be distinguished with the aid of fluorescence microscopy (Felsenfeld, *unpublished data*). At the end of the "negative phase," which lasts 5 to 6 days, short, corkscrew-like metacyclic forms are observed in the celomic cavity of the louse (23, 31, 153). This cavity has no connection with the gut; thus, the borreliae are not found in the feces. Borreliae multiply in the celomic fluid but do not enter the salivary glands, salivary ducts, ovaries, or eggs (153). Hereditary propagation of the borreliae in lice is most unlikely. Borreliae are not injected into human beings with the bite of the infected lice after the organisms have reached the celomic cavity. Lice are very delicate. Antennae and legs are easily broken off, permitting the celomic fluid containing borreliae to contaminate the site of the bite. The louse must be crushed or mutilated to transfer the infection (60). It is believed that most borreliae can cause infection by penetrating through small abrasions of the skin and of the intestinal tract, or even through the intact mucosa (51, 184, 189).

After infestation, the louse remains infectious from the last days of the "negative phase" to about 3 weeks, or its entire lifetime, which is 27 to 50 days. The cycle of borreliae in lice varies with the temperature of the environment (31).

Garnham et al. (128) recommended division of the strains of *B. recurrentis* into two subgroups according to their behavior in lice: (i) long negative phase in the louse (European, North African, and Kenya strains) and (ii) no, or practically no, negative phase in the louse (Abyssinian and Chinese strains).

Experiments with louse-borne relapsing fever are hampered by the unwillingness of human lice to bite readily animals other than monkeys (45). It is possible, however, to feed them on young rodents (95). Feeding experiments have been few in the past and did not include a significant number of rodent species, which are natural hosts of *Borrelia*-carrying ticks. Such experiments could be helpful for the elucidation of the possibility of a wider transmission circle of *B. recurrentis* than that of man-louse-man.

Numerous attempts have been made to establish other borreliae than *B. recurrentis* in lice. Adler and Ashkel (4) found that lice can become infected with *B. persica* but lose them in about 10 days. *B. persica* was not taken up by lice from infected man in other experiments when the Uzbekistan strain of *B. persica* was used. The Tobruk strains disappeared from lice in 2 days (11). Neither was the louse hospitable to the Kashmir strain of *B. persica* (242). *B. crocidurae* and *B. hispanica* were taken up by lice with a long (10 to 12 days) "latent" or "negative phase" (44, 47). Garnham (127) found that lice may

harbor *B. hispanica* and believed that a man-louse reservoir of this *Borrelia* may exist. Sparrow (264) also succeeded in adapting *B. hispanica* to lice; however, her greatest success was with one strain which she considered a mutant.

Many organisms of *B. duttonii* were destroyed in the stomach of lice, but those escaping into the celomic cavity lived and multiplied (147).

It seems that *B. hispanica*, *B. duttonii*, and the borreliae of the crocidurae subgroup become louse-borne more easily than does *B. hispanica*. The environmental temperature seems to affect the process of adaptation. Thus, at 30 C borreliae of the crocidurae subgroup, borne by *O. erraticus sonrai*, were easily established in body lice by Haiserkorn (142).

Heisch and Garnham (149) experimented with lice from an area which was free from relapsing fever (Nairobi) and let them feed on monkeys infected with an East African strain of *B. duttonii*. The lice became infections on the 5th to the 7th day, but borreliae could not be seen in them for 10 days. This "negative phase" was less definitive than in *B. recurrentis* infections. Lice could transmit *B. duttonii* to hedgehogs, mice, rats, and monkeys in the laboratory. These authors found huts in which the inhabitants were infested with lice carrying *B. duttonii*. The same huts were also infested with *O. moubata*, the natural carrier of *B. duttonii*. No changes in the physiology of this *Borrelia* were observed in the lice. These authors, however, did not find evidence for the mutation of *B. duttonii* into *B. recurrentis*.

The monkey louse (*Pediculus longiceps*) is a good host for *B. duttonii*. This *Borrelia* may be transferred from monkey to monkey by *P. longiceps* (145).

It seems that lice may also carry borreliae other than *B. recurrentis*, but the mutation of such strains into epidemic strains has not been observed as yet. The antigenic lability of borreliae in insects is less than in man and animals. This may explain the stability of the borreliae observed in these experiments. If mutation takes place, it is probably the result of repeated "man to louse to man" cycles. Although this is a distinct possibility, to my knowledge it has not been described as yet.

Ticks

Ticks propagating human relapsing fever belong to the Argasidae, never to the Ixodidae. Those of interest in *Borrelia* transmission have been listed or discussed by Anastos (7), Arthur (9), Baker and Wharton (17), Cooley (56), Cooley and Kohls (58), Davis (83), Desportes and Campana (104), Enigk and Gottner (113), Galvão (120), and Hoogstraal (161).

Ticks live in burrows of rodents and other animals, in caves (especially those in which guano is present), in dugouts and trenches infested with rodents, and in human and animal habitats with mud floors. Some feed on animals, including man, indiscriminately; others bite only one or a few species. The feeding takes about 20 to 30 min, or 1 hr or longer, according to the species of the tick. Some produce local analgesia, whereas the bite of others is very painful. Those ticks which are vectors of relapsing fever organisms attach themselves to their hosts for only a short time, usually for less than 1 hr, and their bite is seldom painful. Some of them are night feeders. Whereas lice have to be crushed to transfer *Borrelia*, ticks do not, and a single individual may insect a different person or animal at each feeding.

Ticks may take up blood equal to two to six times their own weight. While they are feeding, saliva reaches the capillaries opened by the bite. Toward the end of the feeding, the contents of the gut are evacuated. Many ticks excrete coxal gland fluid at the end of the feeding. This coxal fluid may be quite voluminous, or only a drop, or may not appear at all while the tick is in contact with its host.

The borreliae are passed to the eggs of the infected female tick, but not all larvae will be infested (5). Nevertheless, this creates a reservoir of *Borrelia* in the tick population. The range of transovarian propagation varies with the species of the tick: from 0.29% in *O. kermsi* to 100% in *O. turicata* (95).

A brief characterization of ticks of known importance in the propagation of relapsing fever follows.

O. erraticus erraticus, the "large type" of *O. erraticus*, is the vector of *B. hispanica*. It is common in Africa from Uganda to the Mediterranean, apparently having followed the path of the Moslem conquest. It lives with burrowing mammals and owls. The larvae feed on rodents and some reptiles, but easily encounter man (46). When mammals are not available, it will bite crabs and frogs. *O. erraticus* feeds for only 15 to 30 min.

B. hispanica includes such strains as the Moroccan (19), the Portuguese (253), the Peloponnesus or Greek (64), and the Normandian (South Tunisia) (251) strains. Atypical Syrian and Algerian strains have also been studied (215, 252). Their insect vector seems to be the same tick, namely, *I. erraticus erraticus*.

In experiments (23), *O. erraticus erraticus* was able to transfer *B. mazzotti* from man to guinea pig, but quite irregularly.

O. erraticus sonrai, also called the "small type"

of *O. erraticus*, is the host of some of the crocidurae group of *Borrelia*, namely, *B. crocidurae*, *B. dipodilli*, *B. merionesi*, and *B. microti* (26); the "large type," *O. erraticus erraticus*, is not hospitable to this group (82). The pathogenicity of these borreliae for man is low (202). High rodent and mild human pathogenicity was stated to be one of the characteristics of this group (30).

There is still some confusion regarding the taxonomy of some ticks belonging to this subgroup and of the borreliae carried by them. *O. normandi* is a strain of *O. erraticus* (9). Thus, it is questionable whether *B. normandi* should be used to designate the *Borrelia* carried by this tick. *O. marocanus* is synonymous with *O. erraticus*. *B. hispanica* var. *marocana* and *B. masouria* are carried by *O. erraticus* and should be classified with *B. hispanica*.

O. savignyi was considered a potential host because of its predominance in some areas afflicted with relapsing fever, like Somaliland where other vectors have not been detected as yet, and in the South of Tunis and Libya, where louse-borne epidemics have originated in the past. There have been too few surveys carried out on *O. savignyi* living under natural conditions to permit a decision as to whether this species is or is not a natural vector.

O. granigeri was found to carry a very mildly human pathogenic *Borrelia*, described by Heisch (146).

O. moubata, the "eyeless tampan," and its four subtypes, namely, *O. compactus*, *O. apertus*, *O. porcinus*, and *O. porcinus domesticus* (283, 284), are at home chiefly in East and West Africa. *O. moubata* carries *B. duttonii*. Walton (281) believed that its original habitats were warthog and porcupine burrows and that it was transferred to human huts by man. It prefers houses where people and fowl live together (282). It lives a few centimeters under the surface in the dust and in cracks of mud floors, particularly near dry places where people usually sit. It has been found in mud and grass walls, even in thatched roofs.

Since *O. moubata* seldom travels by its own volition farther than about 30 yards, transportation by man or animals is necessary to carry it to other localities.

O. moubata and all of its five or six nymph stages suck blood. Adults like to feed every 6 weeks, nymphs every 2 weeks. In the laboratory, it is not necessary to feed adults more often than twice a year. After prolonged starvation, however, borreliae often disappear from its coxal fluid and ovaries (183). The feeding time of *O. moubata* is about 20 to 30 min. About 40 to 60% of the eggs become infected (137). The salivary glands are heavily infested in nymphs and irregularly in adults (277). The feces are free from borreliae (118).

Young adults and nymphs transfer borreliae chiefly through the saliva, whereas older specimens propagate the infection through the coxal fluid.

O. moubata may become infectious as soon as the 5th day after feeding. The first nymphal instar is the most effective transmitter. Since borreliae are being passed on from generation to generation, a mammal reservoir is not necessary to maintain *B. duttonii* and, because of the longevity of this tick, *O. moubata* and its progenies may maintain the infection in an area for a long time (58, 129, 130, 131, 132).

Each subspecies of *O. moubata* usually feeds on only one mammal species (135, 280). Blood is digested very slowly, and the species from which it came can be identified by precipitin tests as long as 7 months after feeding (286). It was proved by this method that burrowing animals are not the source of human infection but that *O. moubata* is the reservoir as well as the transmitting agent of human-pathogenic *B. duttonii* (20, 22, 150).

O. moubata is considered an effective vector. In laboratory experiments, it could transfer *B. crocidurae* but was not very hospitable to it (26); it harbored *B. persica* for many months, and also *B. hispanica*, but not the Kenya type of *B. recurrentis* (128). *B. turicatae* lived in *O. moubata* for 19 months but could not be transferred by its bite. Thus, *O. moubata* has to be considered a tick propagating in nature only *B. duttonii*.

Grün (139) observed that *O. moubata* did not acquire *B. duttonii* infestation when this organism became avirulent after several mouse passages. The reason for this aberration has not been explained as yet.

O. tholozani carries *B. persica*, and probably has several subspecies. It is synonymous with *O. papillipes* and possibly also *O. crossi*, which harbors the Kashmir strain. Babudieri (14) described two *B. persica* strains in Jordan which are, perhaps, carried by different subspecies of *O. tholozani*. The Uzbek strain of *B. persica* causes only mild disease in man (171). *B. persica* is synonymous with *B. sogdiana* and probably also with *B. uzbekistanica*. *O. tholozani* makes its home from the Central Asian USSR through Iran to the Mediterranean. It lives in caves, holes, and burrows of small animals or in human huts. In some localities, it is found near camels and fowl. In Cyprus, it prefers rocky shelters and caves with guano floors (125). In Central Asia it lives in oases and in rodent burrows along the edges of the woodlands of the mountains

(213, 229). It was also found in abandoned piggeries (274). In Kazakhstan, 2% of *O. tholozani* collected in their natural habitats were found to be infected with *Borrelia* (262).

This tick has one larval and three to four, possibly five, nymphal stages which feed at least once on a vertebrate host during each phase of development. The adults feed repeatedly. Transovarian propagation of *Borrelia* in *O. tholozani* has been proved. The incubation time, from the infectious meal to the ability of the tick to transmit *Borrelia*, varies. In adults it may be as long as 1 or 2 months. Moskwin (213) found that the infection is transferred by bite, i.e., through the saliva, and not through the coxal fluid.

O. tholozani adults were observed to harbor *B. persica* for about 3 years and the nymphs for about 5 years (52). About one of every five ticks fed on infected rats or mice acquired *B. persica* (239). In addition to *B. persica* and its substrains, *O. tholozani* was found to be able to transmit *B. recurrentis* and its Moroccan and Berber strains from mouse to mouse in laboratory experiments (180). It carried *B. recurrentis* for 4 months under similar conditions (263), but was unable to transmit it to animals. It hosted *B. hispanica* and *B. microti* after experimental infection (241).

B. babylonensis was isolated from *O. asperus* Brumpt (55). It caused relapsing fever with many circulating borreliae in guinea pigs, thus resembling *B. hispanica*. On the other hand, this *Borrelia* is not transmitted by *O. tholozani* or *O. erraticus*. *O. asperus*, however, will transmit *B. persica*. Thus, it might be more feasible to consider this *Borrelia* within the group of *B. persica*.

O. tartakovskyi carries *B. latyschewii* (262a) and is found in the Central Asian USSR. As a rule, it does not appear in human dwellings; it exists in burrows of rodents and reptiles in dry waste lands, but seldom in the desert. *O. tartakovskyi* exudes coxal fluid not during feeding but some time after it has left its host (27).

O. verrucosus lives in burrows and caves of the semi-desert region of the Caucasus. It has little contact with man. There are four endemic foci in Azerbaijan in which this tick carries *B. caucasica* (237), a human pathogen, which has not been studied satisfactorily as yet.

O. neerensis Pavolovsky, from Turkmenia, prefers more cultivated areas than *O. verrucosus* and inhabits burrows of rodents, birds, and reptiles. It is supposed to carry *B. latyschewii* (230, 238), which is seldom found in man and causes only mild disease (27). It is not known whether *B. latyschewii* from *O. tartakovskyi* is identical with the strain carried by *O. neerenis*.

O. coniceps Canestrini lives in burrows and caves and also in houses with chickens. In Jordan, it has been suspected of transmitting *B. persica* through its saliva (14). *O. coniceps* can be artificially infected with *B. persica*, which it will carry for about 19 months. Transovarian transmission will take place. About 2% to 3% of the ticks become infested by feeding, and about 10% of the guinea pigs develop *Borrelia* infection when infested ticks feed on them (70).

The American relapsing fever *Borrelia* [illegible] were suspected of being tick-adapted *B. recurrentis* (62). Brumpt opposed this theory (53). American *Borrelia* strains apparently did not become tick-borne after the Spanish Conquest or after the settlement of the West commenced, at which time louse-borne *Borrelia* was spread. Tick-borne strains were probably present already in the western mountain ranges of the Americas when immigrants from Europe and from the East arrived.

Among the South American ticks, *O. rudis* (*O. venezuelensis*) is the most important vector. It likes to enter houses and has the habits of bedbugs (233). It carries *B. venezuelensis* (*B. neotropicalis*).

The role of *O. talaje* in relapsing fever is rather puzzling. This tick is found in the West of the Americas, from Canada to Argentina. It often appears near human habitats (42), associates with pigs and rats (79), and seem to transfer *Borrelia* from animals to animals rather than from animals to man (95), although Bates et al. (33) described human infections by *O. talaje*. Some *O. talaje* strains do not bite man (95, 234). The *Borrelia* transmitted by *O. talaje* has not been named as yet, because it is believed that it is identical with that from *O. rudis*. Mazzotti (204, 205) found incongruities between the bionomics of *O. rudis* and *O. talaje* on the one hand, and *B. venezuelensis* infections on the other. *O. talaje* from Mexico and Guatemala transmits a *Borrelia*, called *B. mazzotti* (98), which is not carried by *O. talaje* elsewhere. The literature does not seem to contain reports on attempts to define *O. talaje* subtypes. The problem of South American relapsing fever and its etiology will not be understood until the taxonomy and ecology of the members of the *O. talaje* subgroup are fully investigated.

O. parkeri lives in the West of the United States and in Canada. It inhabits caves and burrows of ground squirrels and prairie dogs. Human infections with its *Borrelia*, *B. parkeri*, are rare, because man does not enter its habitats frequently. *O. parkeri* transmits *B. parkeri* through its salivary apparatus, i.e., by bite. *O. parkeri* cannot propagate *B. venezuelensis*. It will harbor *B. turicatae* for a long time, but is

not effective in transferring it from mouse to mouse under experimental conditions (205).

O. turicata has been found in Canada, the Western United States, Mexico, and in South America. Although it originally favored caves and burrows of rodents, owls, and snakes, it has been found under houses in Texas (42) and seems to begin its domestication in Mexican huts, pigsties, abattoirs, and caves which house goats or sheep. This tick remains alive while starving for 5 years (121). Larvae and nymphs feed for 10 to 30 min, but adults may take as long as 2 days. Coxal fluid is not excreted during feeding, and it does not contain borreliae. *B. turicatae* are transmitted by the bite of the tick (177). *O. turicata* is easily infected with *B. turicatae*; practically all individuals are susceptible and carry borreliae at least to the F5 generation (94). In laboratory experiments, *O. turicata* did not transmit *B. venezuelensis*, *B. hispanica*, *B. duttonii*, *B. persica*, or the Babylonian strain from animal to animal (53, 55, 56, 99).

O. hermsi lives in remains of dead trees (so-called "snags"), with mice, rats, and chipmunks, in the mountains of California, the Northwest of the United States, and Canada (156, 290, 291). Rodents carry it during the winter into summer cabins which are used by hunters and travelers, and where the ticks remain alive for years. *O. hermsi* feeds on mammals but may feed also on other ticks. Wheeler (289) could recover *Borrelia* for 38 days from the gut of *O. hermsi* but found that neither feces nor coxal fluid are expelled during feeding. About 45% of *O. hermsi* found in nature could infect mice. The hereditary transmission rate, however, is low. *O. hermsi* can be infected with *B. venezuelensis* and *B. parkeri* but does not transmit them (205).

The North American species of ticks and the borreliae carried by them are excellent examples of the close relationship between borreliae and their tick vectors. Although borreliae other than the tick-specific borreliae could be transferred to the different ticks, these arthropods were able to transmit only their own *Borrelia* species.

O. brasiliensis harbors *B. brasiliensis* (97) in Brazil; *O. zumpti* harbors *B. tillae* in the Cape Province of Africa (297). The latter is perhaps not pathogenic for man or *Cercopithecus* monkeys (154). *B. queenslandica* from *Rattus villosus* in New Zealand caused relapsing fever in rats and mice. Its tick vector and its human pathogenicity have not been fully investigated as yet (66). It is neccessary to study these organisms more intensively before they can be considered effective factors in relapsing fever.

B. recurrentis isolated in Central Asia could not be transmitted from infected man to man or to rodents by the bite of *O. tholozani* or *O. tartakovskyi* in spite of the survival of the borreliae in these ticks for as long as 134 days (263). Neither could Baltazard et al. (24) transmit *B. recurrentis* from heavily infected infant rabbits by the bite of *O. erraticus*, *O. lahorensis*, *O. turicata*, or *O. parkeri*.

There is a great variety in the mode of living, feeding, and excretory functions of the insect hosts. Attempts to align these factors with the immunology and pathogenesis of the respective borreliae have not been successful as yet.

As seen from the foregoing, a number of *Ornithodorus* species also will carry in the laboratory *Borrelia* types other than those they transmit in nature. The rule of *Borrelia*-tick species specificity, while not unequivocal, comes at least close to being exact. Notable exceptions are the talaje and the latyshevyi subgroups. The results of further investigations may clarify these discrepancies.

According to a hypothesis advanced by Nicolle and Anderson (218), borreliae were originally parasites of small mammals. They were transferred from them to man by ticks, then from man to lice. According to this concept, ticks conserve, and lice propagate, borreliae.

Baker and Wharton (17) stated that *Borrelia* developed with the *Acarinae*. This hypothesis implies that borreliae are primarily the parasites of ticks, invading mammals only by chance and developing into different species with genetic changes of *Acarinae*. It seems that recent reports on the relationship of *Borrelia* strains to the species or subspecies of their vectors favor the second theory.

Unusual vectors, like bedbugs, may occur under certain circumstances. Mechanical transmission as, e.g., by biting flies, is a distinct possibility. Nevertheless, one would go too far to ascribe a large role in the propagation of *Borrelia* to some insects, like mites and bedbugs, which are inefficient transmitters and do not serve as reservoirs of borreliae.

ATTEMPTS TO CLASSIFY BORRELIAE ACCORDING TO SUSCEPTIBLE ANIMALS

Since numerous tick species live with animals in burrows, caves, stables, and even huts, several attempts have been made to classify the members of the genus *Borrelia* according to their presence in wild and domestic animals as well as according to the susceptibility of laboratory animals.

"Natural" Reservoirs

No animal reservoir of *B. recurrentis* has been found as yet. Squirrels could be infected with ease also by administering the Chinese strain per

os or dripping it into the conjunctival sac (78). Bushbabies were sensitive to the Kenya strains (126); gerbils, to the Berberian strain (138).

It is doubtful that these rodents are infected also in nature. A summary of animal reservoirs of tick-borne relapsing fever was presented by Sautet in 1937 (251). Since then, many additional data have been collected. Mooser (212) expressed the belief that of the tick-borne borreliae only *B. duttonii* is primarily anthropophilic, whereas the others are parasites mainly of rodents and insectivorous animals. No animal was discovered as yet to harbor *B. duttonii* under natural conditions. In laboratory tests, dogs, horses, goats, and sheep acquired a light self-limited infection (159). Hedgehogs seem to be immune (186). Chickens injected with *B. duttonii* will harbor borreliae in their circulation for a few days without developing disease (178). The failure of these experiments confirms Mooser's assumption (212) that *B. duttonii* has a life cycle which does not involve mammals other than man.

B. hispanica has been found in rats, jackals, foxes, bats, weasels, hedgehogs, porcupines, dogs, and domestic mice (100, 101, 162, 214, 217, 219). The infection rate varies in rodents from 0.5 to 40% (41).

Pigs, donkeys, and cats have not been found infected, but *O. erraticus*, the tick vector of *B. hispanica*, lives with pigs, and these animals may disseminate *O. erraticus* (214). In laboratory experiments, dogs, procupines, hedgehogs, and bats (186, 217) were successfully infected with this *Borrelia*.

The crocidurae subgroup of *Borrelia* was named after the wild shrew (*Crocidura*) from which the first *Borrelia* belonging in this subgroup was isolated. Shrews, hedgehogs, hamsters, gerbils, bandicoots, wild mice, and sometimes also dogs, bats, horses, goats, and sheep, but not reptiles or birds, are hosts of this subgroup of *Borrelia* (102, 209). Animals which are frequent carriers of the crocidurae subgroup of borreliae in nature may be infected also in the laboratory.

B. persica has been isolated from naturally infected wild mice and rats (14, 25) and may be present in bats. Hedgehogs are susceptible in laboratory experiments, as are sheep (103).

Babudieri (14) believed that there are two strains of *B. persica* in Jordan. One, the rural form, occurs in shepherds, nomads, and road-builders, who often stay in caves with dry and sandy floors. Relapsing fever has its peak among them in the winter. The urban form occurs in windowless houses with earthen floors that are not well maintained. The peak of this infection is in the summer. Babudieri postulated a cycle from the spiny mouse (*Acomys*) or sand rat (*Psammomys*) through *O. tholozani* to man in rural areas, and from domestic rats through *O. coniceps* to man in urban dwellings. These interesting features warrant further investigations.

In the Americas, chipmunks and squirrels (34), burrowing owls (166), monkeys, marmosets, opossums, weasels, armadillos, and bats (79, 111) have been found infected with *Borrelia*. Calves which are tied to bush fence posts in Central America abound with *O. talaje*, and domestic (not ranch) horses around which opossums like to prowl also harbor *Borrelia*.

B. turicatae causes infection in dogs, foxes, cats, pigs, *Microtus mexicanus*, and cotton rats, under laboratory conditions (56, 203).

The search for a wild or domestic animal not routinely used in the bacteriological laboratory as yet for the differentiation of *Borrelia* strains has not been successful to date. Lapiérre et al. (186) achieved some success by employing the European hedgehog (*Erinaceus europeanus*), which is resistant to *B. duttonii*, acquires a usually inapparent disease from the crocidurae subgroup, becomes ill with *B. hispanica*, and develops a severe disease when infected with *B. persica*. The borders between the responses of this hedgehog to borreliae are not sharp and may vary according to the respective strain of the *Borrelia* species employed. Perhaps when more extensive studies of animals harboring borreliae in nature are carried out, when dogs and sheep are added to the series of experimental animals, and when uniform routes and infective dosages are employed, additional laboratory test animals will be found.

Laboratory Animals

Monkeys, mice, rats, guinea pigs, rabbits, and, to a lesser extent, hamsters have been used for laboratory tests with borreliae.

Monkeys gave the best results with *B. recurrentis* after intraperitoneal injection (29, 44). *M. inuus* seemed to develop relapses less frequently than *M. sylvanus* (188). The Tunisian strain killed *M. inuus* (220), but no fatalities were observed in *Macacus* with the World War II strain (80). The Kenya strain killed only 30% of civet monkeys (126). The Manchurian (271) and the Chad Region strains (188) were easily established in monkeys. The incubation period and the number of relapses depended on the size of the inoculum.

B. recurrentis infects mice consistently, but the infection is of short duration (about 3 days) and mild (30, 80). The incubation time depends on the size of the inoculum (31). Young mice are more susceptible (266). Passage through rabbits or monkeys enhances its virulence for mice

(30, 271). Some of the North African strains lost part of their pathogenicity for man after passage through mice (18), and others retained it (21, 266). Strains designated as *B. carteri* and *B. novyi* are also infectious for mice. The Kenya strains of Garnham (126) caused severe borellemia of short duration in mice.

White rats show a short incubation period, and *B. recurrentis* circulates in their blood for 1 week or even longer (20, 30, 126). Sometimes heart blood contains *Borrelia* longer than the peripheral blood. Young rats are more susceptible (265, 271).

It was reported that some West African strains do not infect rats, and the designation *B. berbera* was proposed for them (159).

Guinea pigs appear to be refractory to *B. recurrentis*, including the strains labeled carteri, tonkin, and berbera (134, 159). The Manchurian strains, after having been established in mice, could be transmitted to guinea pigs. They circulated in the blood of these animals for 2 or 3 days (271). Newborn guinea pigs are somewhat more susceptible to *B. recurrentis* than are adults.

Rabbits generally do not become ill after *B. recurrentis* injections but develop antibodies against them. Young rabbits are more susceptible (29, 30, 31). The organisms may persist for 1 or a few more days. Lice usually feed well on newborn rabbits. This method of infection permits a more natural approach in laboratory experiments with these animals. Strains passed through adult rabbits acquire higher virulence for young rabbits (30).

Monkeys, especially *M. inuus*, are highly susceptible to *B. hispanica*. Two or three relapses are often observed in them (48). The infection in mice is short and mild. Rats react in a similar manner (48, 252). Virulence for guinea pigs may fluctuate (252). The appearance of atypical forms and only a few borreliae during remissions in *B. hispanica* experiments with rats may hamper laboratory studies. Most strains, including Moroccan and Algerian, are easily transferred to guinea pigs. Rabbits develop an infection of short duration (48), with few borreliae circulating in their blood.

The crocidurae group is characterized by minimal pathogenicity for monkeys, e.g., *Cynocephalus* (47), and by a greater sensitivity of newborn mice and rats and no reaction or a mild one in adult rats but a serious response in young rats (26, 134). Rabbits are refractory (26).

B. crocidurae, the type strain, causes fatal infection in young rats and in hamsters (44, 45). The Egyptian strain is pathogenic for rats and mice, and causes transitory parasitemia in newborn guinea pigs (106).

B. microti kills young mice and rats. It stays in the peripheral blood of adult mice and rats for 10 to 12 days, i.e., longer than most other Afro-Asian borreliae. The incubation time is about 1 week. It is nonpathogenic for adult guinea pigs and rabbits (95, 240, 241).

B. merionesi causes disease in the Barbari ape (*M. sylvana*) (40) and other monkeys (95). It circulates in the blood of mice for about 2 weeks and kills rats quite frequently. It is nonpathogenic for adult guinea pigs but often fatal for hamsters (40).

B. duttonii is readily transferred to monkeys, causing fatal disease in African species (80). Apes are less susceptible (47). All except the Madagascar strain infect adult mice more readily than newborn mice (134). The infection persists in mice for a long time (80). The same conditions prevail in experiments with rats (128, 134). Guinea pigs are only slightly susceptible to *B. duttonii*, but young animals may acquire the infection. Although adult rabbits are refractory, young may be infected (134).

B. graingeri is only mildly pathogenic (146). Monkeys, guinea pigs, and young rabbits are refractile. This *Borrelia* circulates in the blood of mice and rats only for a few days.

Mice and rats are highly susceptible to *B. tillae*, and young guinea pigs are susceptible to a lesser degree; mild or irregular responses were noted in rabbits (297).

B. persica causes disease in monkeys and mice (3). Guinea pigs are sensitive to most strains of *B. persica*, which is helpful in differentiating it from *B. recurrentis* (3, 4, 103). A hemoperitoneum often develops (53*a*). Rats can be infected (3, 239), but the incubation time is long (103). Prolonged infections in guinea pigs were recorded also with strains from Palestine (10) and Uzbek (274), with an incubation period extending to 6 weeks (103). Numerous relapses were seen in these animals when infected with some of the Tobruk strains (11). Disease in guinea pigs can be evoked also by introducing this *Borrelia* into the anterior eye chamber (3).

Few *B. latyshevyi* circulate in the bloodstream of the infected mice, but relapses can be observed (262*a*). Rats are not sensitive to *B. latyschewii* according to Sofiev (262*a*), which is unusual for a *Borrelia* that is quite pathogenic for mice. Guinea pigs do not react to this *Borrelia* (27, 30, 262*a*). Rabbits show only a transient infection.

B. venezuelensis (*B. neotropicalis*) is able to cause experimental relapsing fever in *Rhesus* monkeys (33). The incubation period in mice and rats varies according to the number of organisms injected. Borrelemia of 1 to 2 weeks' duration

follows, after which the number of circulating organisms diminishes in the blood, but the infection persists in the brain (83, 233). Guinea pigs may be infected with *B. venezuelensis* (92).

B. mazzotti (98, 204) does not infect guinea pigs.

B. turicatae is communicable to monkeys. Mice and rats can be easily infected, and, although the organisms are rare in blood, relapses may occur. *B. turicatae* has been recovered from rat brain but not longer than 6 months after infection. Guinea pigs react according to age and strain (56).

B. hermsii is highly infective to monkeys and easily established in mice and rats (289), but these qualities may vary with the geographic origin of the respective strain. Guinea pigs (92) and hamsters (74) are also sensitive to *B. hermsii*.

B. brasiliensis could be transmitted to mice and guinea pigs (97). Few data are available concerning this strain.

It was shown that, in attempts to classify borreliae according to the response of laboratory animals, the number of the borreliae in the inoculum greatly influences the reaction of the animal host. The incubation time often fluctuates with the dose and the route of inoculation. Host variations may play an important role. Some animals have relapses, and others do not, even if injected with the same species of *Borrelia*.

The experimental methods have not been standardized. Some researchers injected crushed lice or ticks into animals, under the skin or into the peritoneal cavity. It seems that some constituent(s) of the arthropod body enhances the infection. The results of feeding arthropods on animals, infecting them through mucous membranes, injecting infected blood or organs, or introducing infested crushed arthropods, may be divergent, even with the same strain. In addition, one has to consider strain variations. The axiom that no two *Borrelia* strains are completely identical (167) is actually not such an overstatement as it appears at first.

All these factors limit the value of animal experiments. Generally speaking, young animals are more susceptible to borreliae, perhaps with the exception only of *B. duttonii*. Monkeys, mice, rats, guinea pigs, and rabbits are the most common experimental animals. Unfortunately, most authors working with primates used different monkey and ape species. Mice are sensitive to all strains. Some authors prefer rats because, if these rodents are susceptible to a given strain of *Borrelia* at all, the infection will last longer than in mice.

Guinea pig susceptibility to *Borrelia* is a differential diagnostic characteristic, ranging from absence to high sensitivity, with variations according to the strain and the age of the guinea pigs. Rabbits are not helpful in the differential diagnosis of *Borrelia* but can be used for antibody production. Hamsters have not been employed widely in *Borrelia* work as yet. Table 2 summarizes the essential strain differences as established by the experiments related in this section.

PATHOLOGY IN EXPERIMENTAL ANIMALS

The response of experimental animals to *Borrelia* infections depends, among other factors, on their age and metabolism. When dormice were infected with *B. hispanica* or the Uzbek strain of *B. persica* before hibernation, the *Borrelia* disappeared faster than when the animals were kept at room temperature. If dormice were infected during hibernation, borreliae could be recovered for a much longer time (165). This, undoubtedly, is a phenomenon which can be correlated with metabolic changes during hibernation.

The influence of borreliae on the metabolism of infected rats has been studied to some extent and may serve as a clue to further research.

B. recurrentis produced large quantities of lactic acid in the blood of rats, resulting in a higher carbon dioxide respiration and, at the peak of the infection, accompanied by hypoglycemia and depletion of liver glycogen (122).

Severe disease developed in rats that were protein-deficient (140) or thiamine-deficient, or both (141), when they were injected with *B. persica*. There was no change in the relapse rate or in the incubation period. Guggenheim and co-authors (140, 141) believed that the concomitant caloric deficiency was the cause of this phenomenon. Subdeficient diets had no influence on the course of the disease. This observation probably has no bearing on the metabolism of the *Borrelia*, but represents a condition which reduced the host resistance to infections in general.

The fate of borreliae in experimental animals has not been studied to a satisfactory extent. *Borrelia* can be found in the capillaries and sinuses of the organs, especially in the spleen. There is a variation in the intensity of the lesions according to strains. Some authors found that splenectomy in rats has no influence on the infection (20, 22); others observed an increased susceptibility after this operation (168).

The role of the reticuloendothelial system seems to be important, because its blockade may turn otherwise resistant animals sensitive to *Borrelia* (293). When this system was blocked with colloidal iron in rats and mice, the adhesion formation was slower (57). Fixed phages did not

TABLE 2. *Susceptibility of laboratory animals to borreliae*

Borrelia	Mice*	Rats	Guinea pigs
B. recurrentis	Young more susceptible; disease mild; blood positive, 3–5 days	Young more susceptible; disease mild; blood positive, 1 week or longer	Some young susceptible; adults 0
B. hispanica	Young more susceptible; disease mild; blood positive, 2–5 days	Young very susceptible; disease mild; blood positive, 1–2 weeks	Disease may be severe; Many borreliae in blood
Crocidurae subgroup	Young very susceptible; long disease	As in mice	Usually 0
B. duttoni	Old more susceptible; long disease	Old more susceptible; long disease	Young more susceptible; long disease
B. persica	Long incubation; mild infection	As in mice	Very severe disease
B. latyshevyi	Few borreliae in blood but animals sick	0 (?)	0
B. venezuelensis	Disease mild; blood positive, 1–2 weeks	As in mice	Susceptible
B. mazzottii	Disease mild; blood positive, 1–2 weeks	As in mice	0
B. turicatae	Disease mild; few borreliae in blood	As in mice	Only young susceptible
B. parkeri	Disease mild; few borreliae in blood	As in mice	Only young susceptible
B. hermsi	Susceptible; variable picture	As in mice	As in mice
B. brasiliensis	Disease mild; blood positive, 1–2 weeks	As in mice	As in mice

* Mice, especially young, are susceptible to all borreliae. Monkeys are susceptible to practically all strains.

respond to live borreliae, but they ingested dead borreliae (181). Polymorphonuclear cells were seen to take up fragments of borreliae (269), but they do not seem to play a role in the pathogenesis of relapsing fever. Borreliae will adhere to them, even if their cytoplasm has been damaged (3). It seems that shortly before the crisis, borreliae roll up and are taken up by the endothelial cells of the spleen, liver, and bone marrow (269). Surviving borreliae remain in these organs and in the brain until the next relapse.

Borreliae are probably destroyed in the body by antibodies, not by phagocytosis (165, 181). In the brain, they seem to be better protected from antibodies because of the anatomy of the blood supply (196).

The brain changes have been studied with the novyi strain of *B. recurrentis* in white rats (201). Borreliae were present in the capillaries of most parts of the brain. There were no changes in the nerve cells, but an intense microglial reaction was present in the cortex. It is unknown whether there is a relationship between this reaction and the strong neurotropism of some (e.g., *B. persica*) and the weak neurotropism of other (e.g., *B. turicatae*) strains.

While erythrolysis is not observed in routine tissue preparations, Robertson (245) proved that all laboratory animals successfully infected with borreliae show an anemia which is essentially microcytic and hyperchromic with a strong reticulocyte response.

Of the sensory organs, the eyes suffer most frequently. Inoculating the eyes of rabbits was proposed by Blanc et al. (39) for the differential diagnosis of *Borrelia* strains. In his experiments, *B. hispanica* produced lesions similar to syphilitis keratitis; *B. duttonii* and *B. merionesi* gave a different histological picture. Moreover, *B. duttonii* caused a strong local reaction. Unfortunately, only few *Borrelia* strains were used in these experiments.

Indeed, the data on the relationship of cellular response and immunology in borreliasis are meager.

IMMUNOLOGY

Agglutinins

Sera prepared against *Borrelia*, especially when used in lower dilutions, have a tendency to give cross-reactions not only with other *Borrelia* species but also with *Treponema* (250).

Hindle (158, 159) called attention to the complexity of the agglutinating antigens and considered this test technically difficult. Autoagglutination of borreliae is common, and is often seen in the blood of man and monkeys during disease.

Stein (268), however, achieved satisfactory results with *B. recurrentis* by use of saponin-treated rat blood in macroscopic agglutination tests. Adler and Ashbel (3) found significant agglutinating antibody levels in rats infected with *B. persica* after the attacks. Balteanu et al. (32) studied agglutinin titers in man and observed that they rise after attacks, reaching their maximum during convalescence.

Because of the inability of *Borrelia* to grow in artificial media and the difficulties involved in their separation from blood, animal organs, or from the fluids of the developing chick embryo, it cannot be expected that the study of *Borrelia* with the aid of the classic agglutination test will achieve any degree of popularity.

Adhesion Phenomenon

Hindle (159) and Schuhardt (254) surveyed the literature on the adhesion phenomenon. Little adhesion is observed during the early stages of infection but the phenomenon becomes increasingly apparent as the disease progresses. Adhesion is observed in animals and man just before the end of the attack, when borreliae are ready to disappear. It is followed by fragmentation of the infective organisms. The adhesion test is feasible also when only a few borreliae are present in the blood. Mooser (211) found that, in the adhesion phenomenon, borreliae form aggregates or cling to red and white blood cells, to slides, cover slips, and to bacteria like *Escherichia coli* added to the blood. If red blood cells are treated with homologous anti-red blood cell sera, there will be no adhesion of borreliae to red blood cells. Adler and Ashbel (3) saw borreliae clinging to the nuclei of leukocytes, but only after the protoplasm of the white blood corpuscles was destroyed.

The technique of the adhesion test consists of mixing fresh serum with an equal amount of a suspension of borreliae and of an *E. coli* suspension, incubation for 20 min at 30 C, and reading the result with dark-field illumination. If old serum is used, the addition of complement is necessary. This test was developed before more modern techniques for such reactions, e.g., the use of red blood cells, were introduced. The adhesion test is no longer used for diagnostic work because of its variations in patients.

Complement Fixation

Eidmann et al. (112) prepared antigens from rat blood and used them against human and mouse sera in complement-fixation tests. *B. hispanica* was the test organism. Only low antibody levels were observed with 1:10 and 1:80 dilutions of the antigen. Stein (268) achieved satisfactory results by use of the saponin-treated blood of rats infected with *B. recurrentis* or *B. hermsii*, containing at least 80 borreliae per oil immersion field. Wolstenhome and Gear (294) inoculated *B. duttonii* into 7-day-old chick embryos through the air sac with 0.4 ml of heart blood of experimentally infected mice. After 1 week at 37 C, the chorioallantoic vessels were opened and allowed to bleed into the allantoic fluid; 0.1 ml of this mixture was used for further egg inoculations. After 10 weekly passages, phenol-saline was added, and, after centrifugation, was used as the antigen in dilutions of 1:25 and 1:100. Positive reactions with the first of these dilutions were considered diagnostic. Syphilitic sera did not give positive reactions with this antigen, but typhus sera did. This test is seldom used in diagnostic laboratories because of the difficulties encountered in the preparation of antigens from some borreliae which are not easily adapted to developing chick embryos.

Borreliocidal Antibodies

The borreliocidal activity seems to be identical with anti-*Borrelia* cytolysin (114). Belezki and Umanskaya (35) believed that this antibody is most important in the defense against *Borrelia*.

Arboni (8) found that *B. duttonii* survived in the presence of normal guinea pig, rabbit, pigeon, fowl, and horse sera. It was killed by normal cattle, goat, and sheep sera. Sera from pigs gave divergent results. This effect disappeared after the inactivation of the sera. Oag (226) observed that the blood or the serum of mice, fowl, and fowl embryos destroyed *B. duttonii* in vitro, but only the blood or the serum of fowl killed this organism in vivo.

Balteanu et al. (32) found high lysin titers after attacks in man, especially in convalescents. Ballif et al. (17*a*) examined 3,700 human sera. The highest titers, to 1:20,000, were observed during the crisis of the first attack. The lysins were found 10 months and even longer after the beginning of the disease. Ballif et al. found the lysins to be relatively stable in vitro but observed that treatment of the patients interfered with their formation. Pfister (232) verified this in experiments with *B. hispanica*. The sera of patients and guinea pigs showed lysins for several months, but the results were inconsistent and could not be utilized for a retrospective diagnosis.

Lysin-fast strains may develop (59, 272). Perhaps borreliae form an antilysin during their sojourn in the body (272). Lysin and antilysin undergo considerable variations during relapses.

The usual method of testing for lysins is to mix serum and borreliae, incubate at 37 C for 2 hr, and observe the result under a microscope. It is possible to set up the test directly on slides.

Immobilizines

The immobilizing antibody does not represent a large molecule, because it could not be sedimented by centrifugation at 80,000 rev/min (194). According to Calabri (61), it is related to the β- and γ-globulins.

Immobilizines may or may not be related to lysins (254). Levaditi et al. (194, 195) proved that man, mice, rats, and guinea pigs possess immobilizines for a long time after infection. They appear in the mouse blood even while live organisms are still present in the brain of the animal. *B. duttonii* recovered from the brain, spleen, and blood of experimental animals during the latent phase of the infection was resistant to immobilizines. This resistance was not lost in several animal transfers. Levaditi et al. stated that this resistance develops under the action of antibodies on the *Borrelia* and that it is similar to the immobilizine observed in *Treponema* infections. Ranque et al. (243) expressed a similar opinion.

Vaisman and Hamelin (276) studied immobilizines against *B. duttonii* and *B. hispanica*. They observed that immobilizines were highly strain-specific. This was confirmed in our experiments with *B. parkeri* and *B. turicatae* (Felsenfeld, *unpublished data*).

Lysins and immobilizines seem to present valuable tools in *Borrelia* studies because of their strain and relapse-variant specificity. They are probably the most important antibodies which modify borreliae in animals and eliminate them from the body.

Cross-Protection Tests

Beck (34), Johnstone (167), and Schuhardt (254) reviewed the difficulties encountered in the evaluation of animal-protection tests in *Borrelia* infections. Immunity to one strain of a *Borrelia* species does not convey resistance against another strain of the same species in many instances. Dubois (110), for example, found three immunologically different *B. duttonii* strains within an area of a few square miles in Ruanda. Similar observations were made in Iran with *B. persica* (239). Colas-Belcour (81) reported that mice protected against reinfection with the same strain were only slightly refractory to other strains of the same species of *Borrelia* belonging to the crocidurae subgroup. Of three *B. turicatae* strains, only two showed cross-protection (176). Six *B. duttonii* strains collected in Tanganyika displayed little cross-immunity. Mice infected with the strain marked "B" were refractile to strains "C" and "D," but not vice versa. Geigy and Burgdorfer called this phenomenon a "one-sided immunity," probably a type of premunity (133). Similar observations were made by Addamiano and Babudieri in Jordan (2), using two strains, Irbid and Husu. They called the phenomenon an "asymmetric immunity." On the other hand, the Kenya type of *B. recurrentis* gave cross-protection in bushbabies against *B. duttonii* but not regularly in monkeys (128), showing overlapping cross-immunity between representatives of two *Borrelia* species. The novyi strain of *B. recurrentis*, and *B. duttonii*, however, were shown to differ from the three Californian *Borrelia* types by this test (83).

Schuhardt (254) concluded that reciprocal immunity is not always the rule and that even the variants of a single strain collected from the same patient at different relapses do not show complete cross-protection.

Because of these difficulties, the cytolysin test and the interpretation of the Pfeiffer phenomenon are often preferred for establishing the presence of cross-immunity.

Relapse Strains and Phase Variation

During the first attack of relapsing fever, man and animals develop a relative immunity against the infective agent. This immunity may be only of short duration (1). At the same time, some characteristics of the causative *Borrelia* may be changed under the influence of the antibodies produced against them, and so-called relapse strains develop (248, 249). These phenomena have been discussed at length by Hindle (159, 160), Johnstone (167), Russell (249), Schuhardt (254), and Stavitsky (267).

According to Burrows (59), relapse strains occur because of the antigenic instability of *Borrelia*. Relapse strains of *Borrelia* acquire divergent immunobiological reactions which may remain fixed when they are transmitted from one animal species to another but as a rule disappear in the insect vector (10). Relapse strains seem to be limited in the extent of their variability but usually develop specific individual characteristics. Passage through animals may change their serological properties but at the end all final relapse strains react in the same or in closely similar ways (167). It seems that in relapsing fever one deals with a cyclical disease due to a cyclical agent. This holds true also for trypanosomata which behave like borreliae in many respects.

The plasma collected during the crisis of an attack contains antibodies which delay the development of borrelemia when the plasma is injected together with *Borrelia* into other animals (61). This activity is not destroyed by preserving the plasma at 4 C for 4 days. If the relapse is terminated artificially, by the use of a drug, unusual variations in the antibodies and in the *Borrelia* may be observed.

The virulence of the relapse strains is frequently lower than that of the original strain (175). Virulence, however, is a relative attribute. Experimenting in mice and with *B. recurrentis*, Kroó (182) found that strains of high virulence and good immunizing qualities produced an acute infection, whereas those with low virulence gave rise to recurring attacks. Apparently the faster formation of antibodies in infections with organisms carrying more effective antigens terminates the disease more rapidly.

Schuhardt and Wilkerson (255) saw multiple *Borrelia* variants developing in rats infected with *B. turicatae*, even after injecting only one single *Borrelia* organism. Thus, relapse is an immunological phenomenon resulting from the inherent capacity of the *Borrelia* to undergo antigenic variations.

The number of antigenic variants differs from strain to strain. Cunningham (89) described a variation of the carteri strain of *B. recurrentis* from a phase called "A" to another called "B" with a tendency to reverse. Later he and his co-workers (90, 91) found additional phases and concluded that phase A appears in the first attacks, phase B in the first relapses, and phase C irregularly but mostly in the second relapse. D to H were found in the second relapse and I in the first relapse. A, B, D, and E seemed stable and definite; F was rare, persisting through many passages but usually supplanted by B. G was related to A; C, D, and E, to B. In man, mostly A and B were found. H and I were recovered from prolonged attacks, usually together with B. Phase variation was observed also in ticks. As many as nine *Borrelia* variants were found in a single tick (90). Mekeney (207) reported six antigenic *Borrelia* variants in rats when studying the Chinese strain of *B. recurrentis*. Russell (248), experimenting with *Cricetomys gambianus*, observed phases A and B, later also C. Since immune bodies were transferred from animal to animal during the inoculations, she considered it necessary to collect strains in the beginning of the relapses when there is less antibody activity. *Borrelia* strains could readapt themselves antigenically several times but the number of readapted organisms was limited in these experiments.

The antigenic schedules suggested by Cunningham et al. (90, 91) did not gain popularity, because there are differences in the immunological aspects of each individual *Borrelia* strain.

Reinfection

The literature contains reports concerning surprisingly short periods of immunity in man. Simmons (260) stated that reinfection can take place in 2 months; others (292) said that this may happen in 40 days to 6 months. Apparently, a revaluation of recurrence versus reinfection is desirable also in relapsing fever (115).

In areas where relapsing fever is endemic, the disease is usually more severe in newcomers (191, 284) because of the early experience of the local inhabitants with borreliae, but little is known about the immunological responses in such populations.

Immunity in animals seems to last relatively long. In guinea pigs, for example, premunity for 2 years was observed (256). On the other hand, hooded monkeys could be reinfected with the Tripolitan strain of *B. hispanica* after only 6 to 9 months (221). Superinfections in nonimmune animals gave interesting results. When hamsters and Rhesus monkeys infected with *B. recurrentis* were superinfected with the Chinese strain of *B. recurrentis*, the monkeys did not respond at all and the hamsters developed only mild infections (75). When mice infected with *B. dutlonii* were superinfected with *B. crocidurae* and *B. hispanica* simultaneously, the period of circulation of the borreliae in the blood was prolonged, but there was no change in the course of the infection (172).

Clearly, much additional information is needed in this field.

Hereditary Immunity

Nohira (224) showed that the offspring of female rats immune against the Manchurian strain of *B. recurrentis* were immune for about 60 days against the same strain. Nohira claimed that the antibodies were transferred through the placenta. If a woman becomes ill with relapsing fever during pregnancy, however, abortion terminates the life of the fetus. Antibodies were found in the milk (248). No further data are available on this important problem.

Interference

B. recurrentis and *B. hispanica* did not interfere with *Spirillum minus* infections in rats (199, 114).

Tests used for the diagnosis of syphilis may become positive during relapsing fever. The

Wassermann reaction was positive in 30% of the patients in China (71) and in one case in the United States (216), but it appeared to be positive only for a short period (244). The Kahn reaction was positive in 15% of patients in China (71), transiently positive in 4 of 24 cases in Cyprus (107), and negative in sick persons in Kenya (128).

When *B. recurrentis* was injected into mice harboring *Leptospira icterohaemorrhagiae*, the course of the infection did not differ from that observed after the injection of *B. recurrentis* alone into healthy mice (114).

B. duttoni did not alter the course of coxsackie B virus infection in mice (192) nor that of *Rickettsia* (*Coxiella*) *burnetii* in guinea pigs (38).

R. burnetii did not interfere with *B. hispanica* infection in rats (199).

The relationship of borreliae to typhus fever is an interesting problem. In man, agglutination tests with OXK antigens were positive in relapsing fever to high titers in Africa, but with OX19 in only a few instances (170, 296).

It is not known whether borreliae interfere with rickettsiae in the louse. Observations during World War I showed that epidemics caused by these agents increase simultaneously but decrease separately (65). In an outbreak in Addis Ababa (170), epidemic relapsing fever disappeared sooner than typhus. In Yugoslavia, after World War II, relapsing fever was more common in localities where epidemic typhus was rare (258). Perhaps the various *Borrelia* and *R. prowazeki* strains have different influences on each other.

A close relationship between borreliae and some tissue and blood protozoa has been reported.

Borrelia infections activated clinically latent *Leishmania donovani* infections in man (87).

Trautmann (273) was the first to experiment with *Trypanosoma* and *Borrelia* in the same animal. Rabbit serum against *B. duttoni* immobilizes but does not agglutinate *T. brucei* and vice versa.

B. crocidurae and *B. duttonii* extended the life-span of mice inoculated with *T. gambiense* (187). *B. hispanica* was less effective than *B. duttonii* in mice infected with *T. rhodesiense* (185).

Interesting chronological observations were made by Vincent (279). When *T. annamense* was inoculated into white mice together with *Borrelia*, the incubation time was the same as when each organism was administered separately, i.e., 3 days for *Borrelia* and 2 to 5 days for *Trypanosoma*. The trypanosomata multiplied slowly, and the animals did not die in 5 to 9 days, as usual, but trypanosomata greatly decreased from the 7th day on. Then borreliae increased in number and this infection became petulical. After that, trypanosomata appeared in greater numbers and the animals died in 30 to 40 days.

Carminati (67) experimented with three strains of *Trypanosoma* and *B. duttonii*. *B. duttonii* retarded *T. brucei*, *T. gambiense*, and *T. equiperdum* infections in mice. Interference occurred only when large numbers of *Borrelia* circulated in the blood. 2,3-Dimercaptopropanol, dextrose, vitamin C, and cytochrome did not affect this antagonism. There were no anti-*Trypanosoma* antibodies in the sera of animals treated with *B. duttonii* alone. In animals infected with *Borrelia*, lesser susceptibility to *Trypanosoma* was noted also after the *Borrelia* disappeared from the blood.

The mechanism of the interference of *Borrelia* with *Trypanosoma* is not well understood as yet. These genera are biologically related. Since some borreliae, like *B. persica*, do not display interference, it is difficult to accept the theory that this is merely the result of a genetic relationship between *Borrelia* and *Trypanosoma*. Vincent's theory (279) that macrophage participation is the cause of interference is not borne out by the histological findings seen in both infections. Since the biochemistry of *Borrelia* and *Trypanosoma* antigens and antibodies has not been worked out completely as yet, one may hope that a better understanding of the problem will result from such studies.

Galliard et al. (123) utilized the phenomenon of antagonism for the differentiation of tick-borne borreliae. They recommended that mice be infected with the unknown or unidentified *Borrelia* strain; then, at the height of the first attack, that *T. brucei* be injected. If mice survive for a long time, and if some even appear to be cured, the unknown strain may be *B. duttonii*, *B. micrati*, *B. merionesi*, or *B. crocidurae*. If there is no protection and the mice die within 1 week, the organism may be *B. hispanica* or *B. persica*. If there are variable results, *B. turicatae* has to be considered.

The observations of Galliard et al. are interesting also from the genetic point of view. Since borreliae are adapted to their tick vectors, the possible relationship between the subspecies of insects and the *T. brucei* inhibiting activity of the respective *Borrelia* strains may deserve further study.

Therapeutic Sera and Vaccines

The use of convalescent sera in the treatment of relapsing fever was recommended by Sergent (256). Baltzanu et al. (32) reported chills and even collapse in about 75% of their patients treated with such sera. When the serum was

administered intramuscularly, the reaction was less violent. These authors believed that it is a Herxheimer type of reaction, due to the sudden disintegration of the borreliae.

Sergent (256) also reviewed the problem of vaccination against *Borrelia* and concluded that neither heat-killed nor bile vaccines are effective. Heat-killed vaccines protect only against infections with the strain from which they were prepared. Lysins develop in the vaccinated animals, which become refractile to small intracutaneous doses of the homologous strain.

PRACTICAL LABORATORY DIAGNOSIS

The laboratory diagnosis of *Borrelia* in human infections is hampered by the paucity of the organisms in the blood stream in some types of relapsing fever, especially in children. Borreliae disappear from the circulation or their number greatly diminishes shortly before the crisis. Thus, inoculation of young mice with blood or spinal fluid, or both, may be the only means of demonstrating them (43).

For staining of thin and thick smears, the Giemsa stain with gentle heating for 4 to 5 min is the most convenient method, or the Wright stain with a subsequent short (10 to 30 sec) staining with 1% crystal violet can be used.

Xenodiagnosis was suggested as a laboratory method, but it gave unreliable results (23).

The determination of lytic and immobilizing antibodies may be helpful but difficult to interpret in relapses.

SUMMARY

Borreliae causing relapsing fever can be divided into louse-borne and tick-borne groups. The recommended classification of the strains is according to their specific arthropod vectors. Morphologically, the species do not differ from each other. Little is known about their metabolism. Culture methods are not satisfactory for the propagation of borreliae. They grow in developing chick embryos but are not easily adapted to this host for long-range cultivation.

A considerable number of scientific experiments have been reported in the literature concerning the artificial establishment of borreliae in insect species other than those by which the respective strains are carried in nature. Since many authors used different techniques and worked with relatively few strains, the results are confusing. It seems, however, that tick-borne borreliae remain tick-borne, and perhaps only exceptionally become louse-borne and epidemic. Borreliae, however, seem to have originated and developed with ticks, and to have invaded small mammals and become hemanotropic only after a long period of adaptation. Their propagation and geographic distribution depend on the biology of their vectors. Animal reservoirs do not seem to play so large a role in the epidemiology of relapsing fever as was believed in past decades.

Immunological studies of *Borrelia* revealed the importance of immobilizing and lytic antibodies. There are extensive phase variations and mutations in infected animals and man, yielding so-called relapse strains. This further hampers the classification of the isolated types.

The practical laboratory diagnosis is based on mouse inoculation and on blood-smear studies.

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